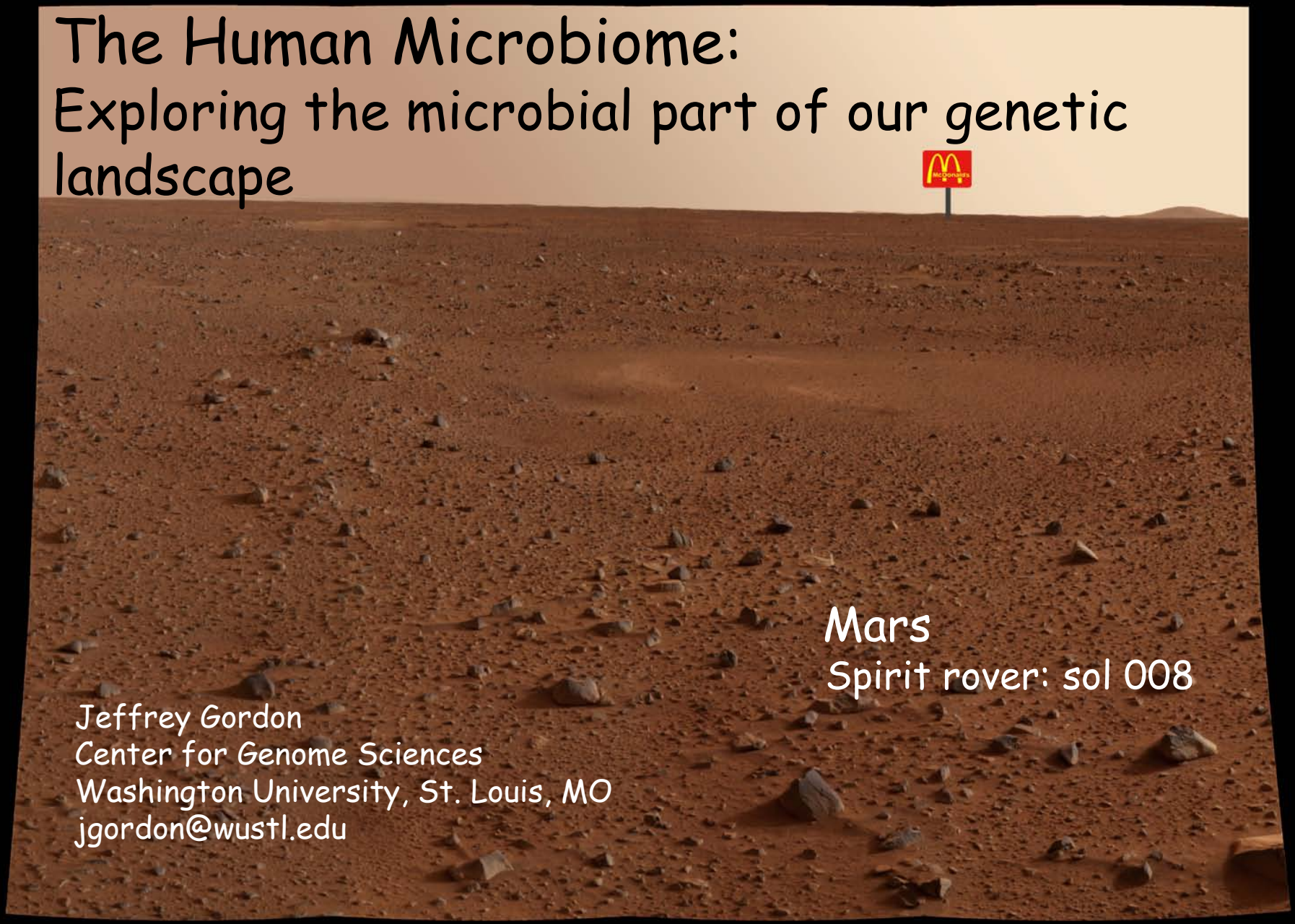
The Human Microbiome:
Exploring the microbial part of our genetic landscape



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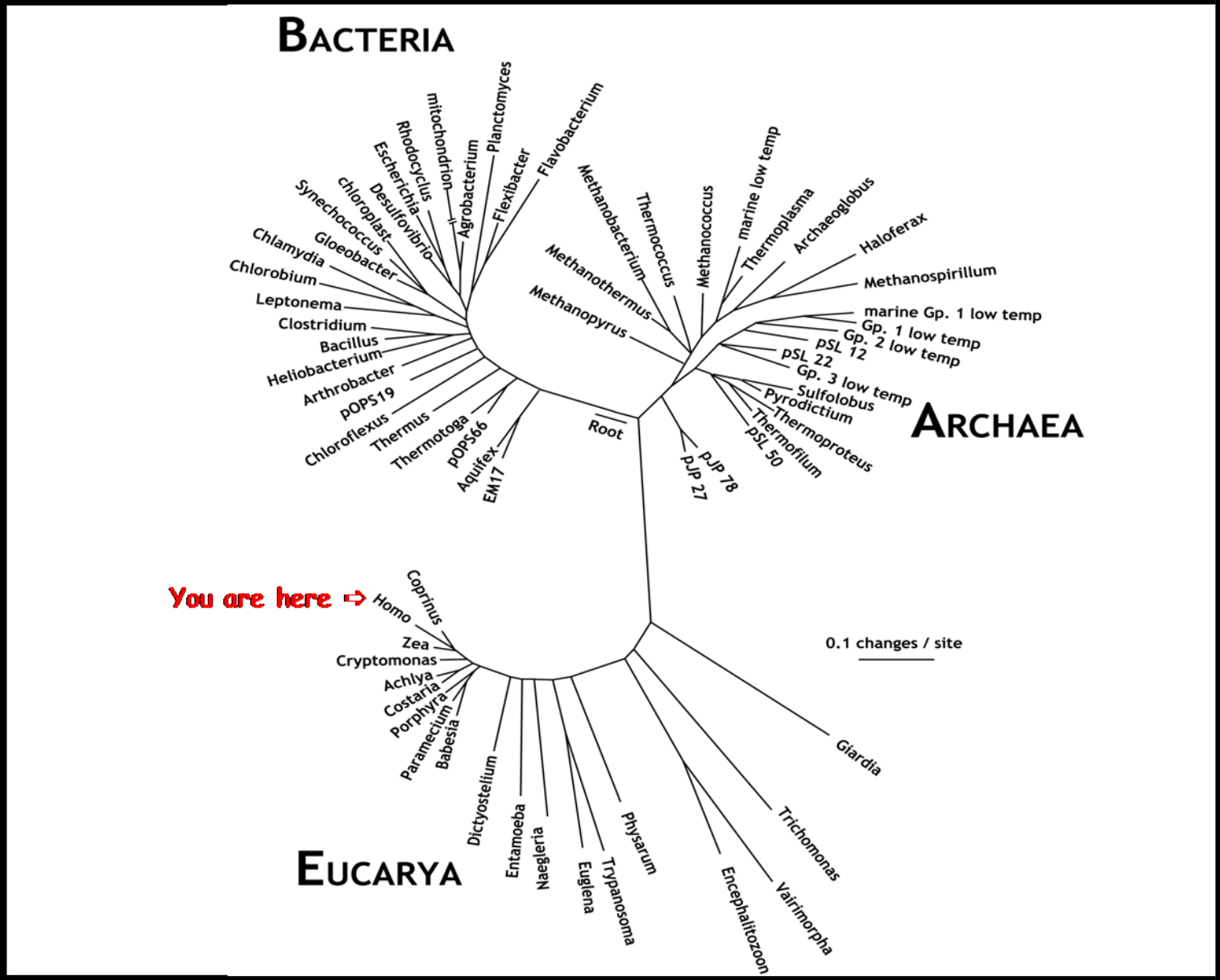
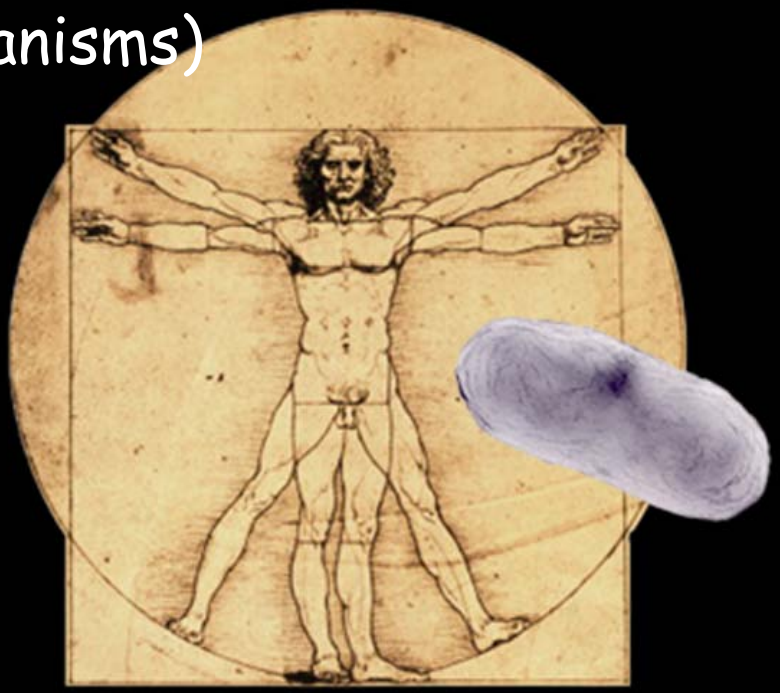
Tree of Life


BACTERIA
mitochondrion
Planctomyces
Agrobacterium
Flexibacter
Flavobacterium
Rhodocyclus
Escherichia
Desulfovibrio
chloroplast
Synechococcus
Gloeobacter
Chlamydia
Chlorobium
Leptonema
Clostridium
Bacillus
Heliobacterium
Arthrobacter
pOPS19
Chloroflexus
Thermus
Thermotoga
pOPS66
Aquifex
EM17
Methanobacterium
Thermococcus
Methanococcus
marine low temp
Thermoplasma
Archaeoglobus
Haloferax
Methanothermus
Methanospirillum
Methanopyrus
marine Gp. 1 low temp
Gp. 1 low temp
Gp. 2 low temp
pSL 12
pSL 22
Gp. 3 low temp
Sulfolobus
Pyrodictium
Thermoproteus
Thermofilum
pSL 50
ARCHAEA
Root
pJP 78
pJP 27
You are here
Homo
Coprinus
Zea
Cryptomonas
Achlya
Costaria
Porphyra
Paramecium
Babesia
Dictyostelium
Entamoeba
Naegleria
Euglena
Trypanosoma
Physarum
Encephalitozoon
Vairimorpha
Trichomonas
Giardia
EUCARYA
0.1 changes / site

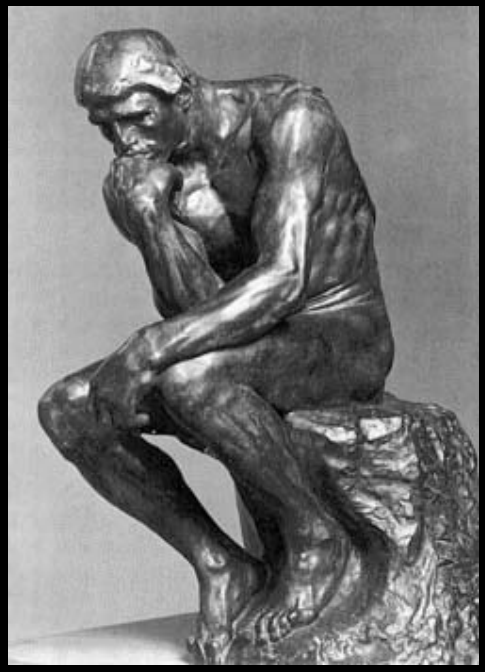
Extended view of ourselves as a lifeform

- We are composite of species: a 'supra-organism'
- Our microbial census exceeds the total number of our own human cells by ~10 fold
- Our largest collection of microbes resides in the intestine (~10-100 trillion organisms)
- The aggregate genomes of these species = microbiome
- The microbiome is an integral part of our genetic landscape (our 'human meta-genome')

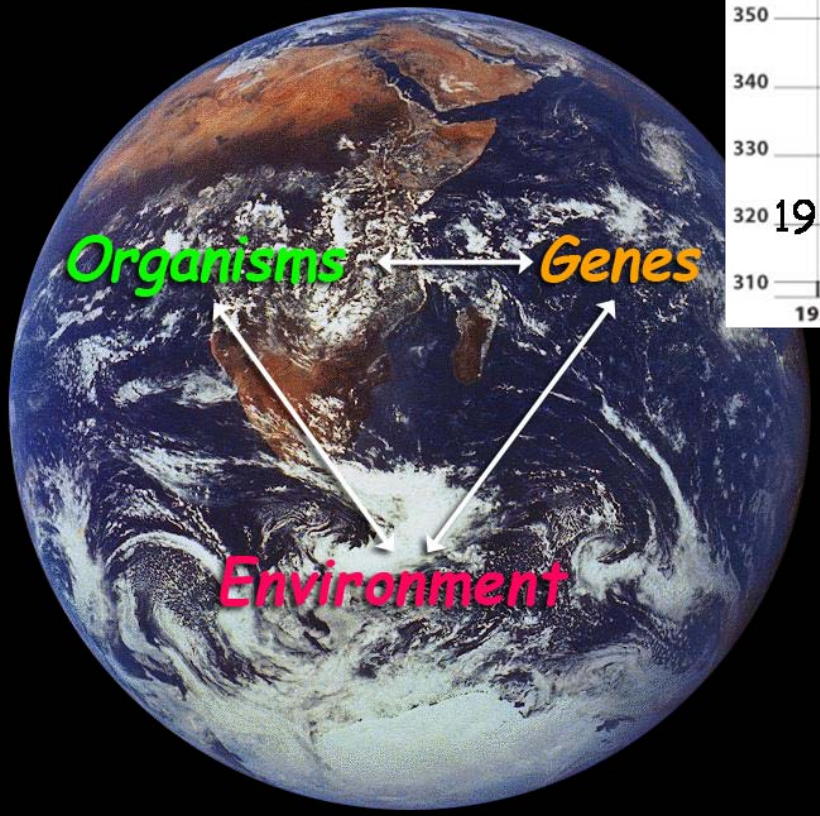


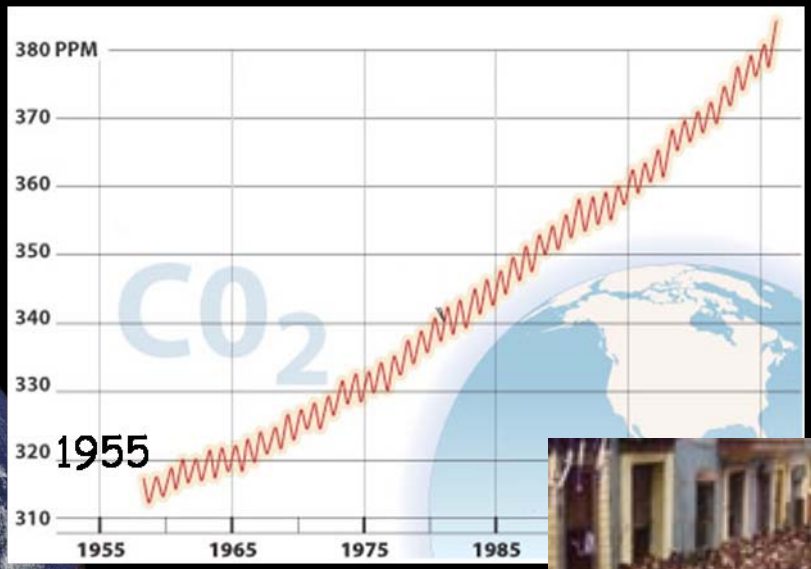
Our Human Microbiome: Questions to Ponder

- Should differences in our microbiota and microbiome be viewed as features of our biology that are profoundly affected by both our *H. sapiens* genotypes and by our individual 'environmental' exposures?



How is our microbiome evolving (within and between individuals) over varying time scales as a function of our changing diets, lifestyle, and biosphere?





A quick primer

Analyses of diversity:

- Alpha diversity (how many types of sequences in a sample) or beta diversity (how different types are distributed among samples)
- Qualitative or quantitative
- Phylogenetic or taxon-based

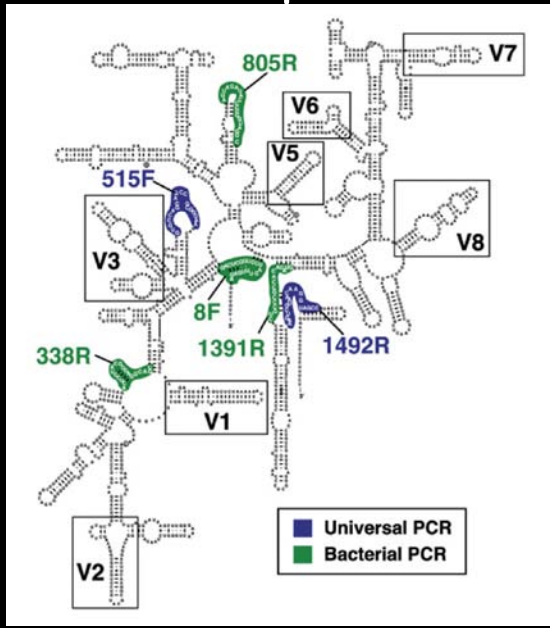
The influence of sex, handedness, and washing on the diversity of hand surface bacteria

Noah Fierer[a,b,1], Micah Hamady[c], Christian L. Lauber[b], and Rob Knight[d]

Rob Knight
University of Colorado, Boulder

A quick primer

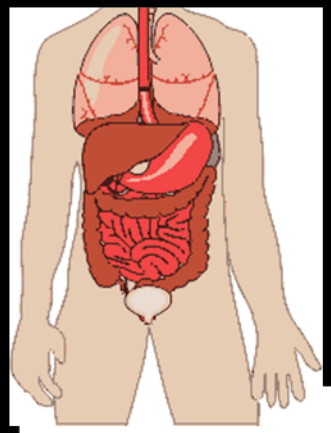
- 16S rRNA gene present in all Bacteria and Archaea
- Gene contains highly conserved and more variable regions
  - amplify 16S rRNA genes using primers directed at conserved regions but flanking variable regions
  - align gene sequences, resolve phylogenetic relationships at different depths
- Most 16S rRNA sequences come from previously undescribed microbes
  - taxa operationally defined based on sequence similarity: e.g., members of a species share ≥97% 16S rRNA sequence identity



Fierer *et al.*, PNAS 105:17994 (2008)

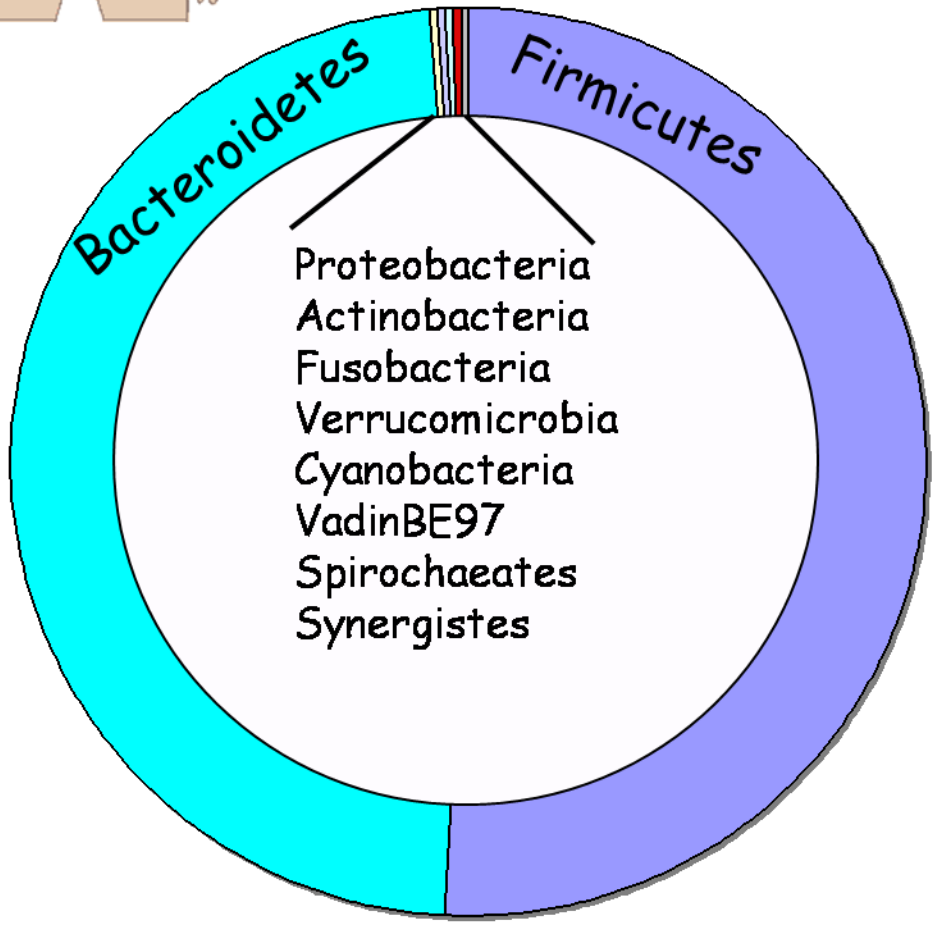
Hands

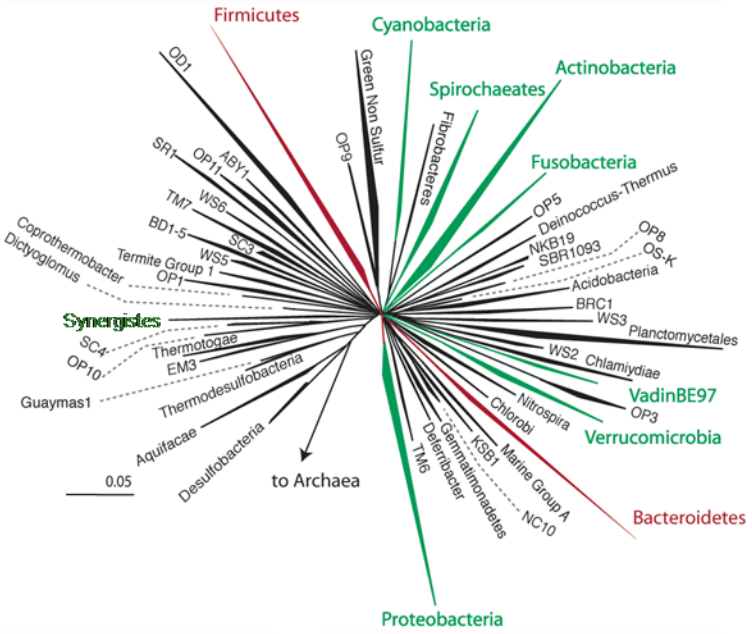
- 51 college students (after exam): dominant and non-dominant hands
- barcoded multiplex pyrosequencing, targeting V2 region of bacterial 16S rRNA gene
- surprising degree of diversity >150 species level phylotypes/palm; 4742 unique phylotypes observed
- Pronounced intra- and interpersonal variation
- Hands from same individual share only 17% of species-level phylotypes
- Women have higher diversity than men
- Community composition significantly affected by handedness, time after handwashing
- Study illustrates challenges inherent in defining what constitutes a healthy bacterial community



GUT COMMUNITY:
16S rRNA sequence-based studies of the adult human distal gut microbiota

e.g., Eckburg *et al.*, (11,831 sequences); Ley *et al.*, (18,348)





10 of 70 described divisions of Bacteria
Also methanogenic Archaea:
Methanobrevibacter smithii
Methanosphaera stadtmanae

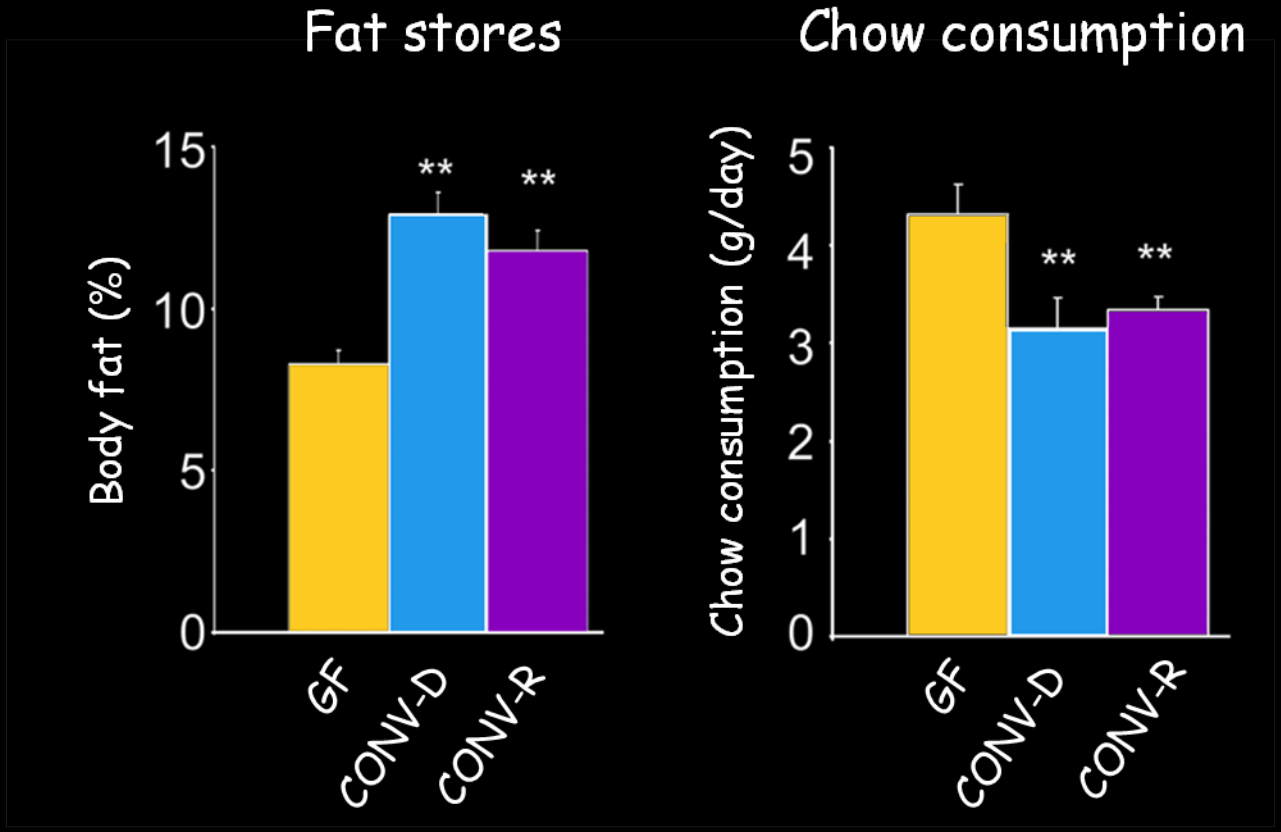
Hypothesis

- Diet is a prominent factor that shapes the gut microbiota
- There is a dynamic interrelationship between diet, gut microbial ecology and energy balance
- Variations in gut microbial ecology and the gut microbiome that affect the efficiency of harvest of nutrients and energy from a particular diet contribute to risk for obesity (and malnutrition)

A rapid increase in adiposity occurs with colonization of germ-free mice

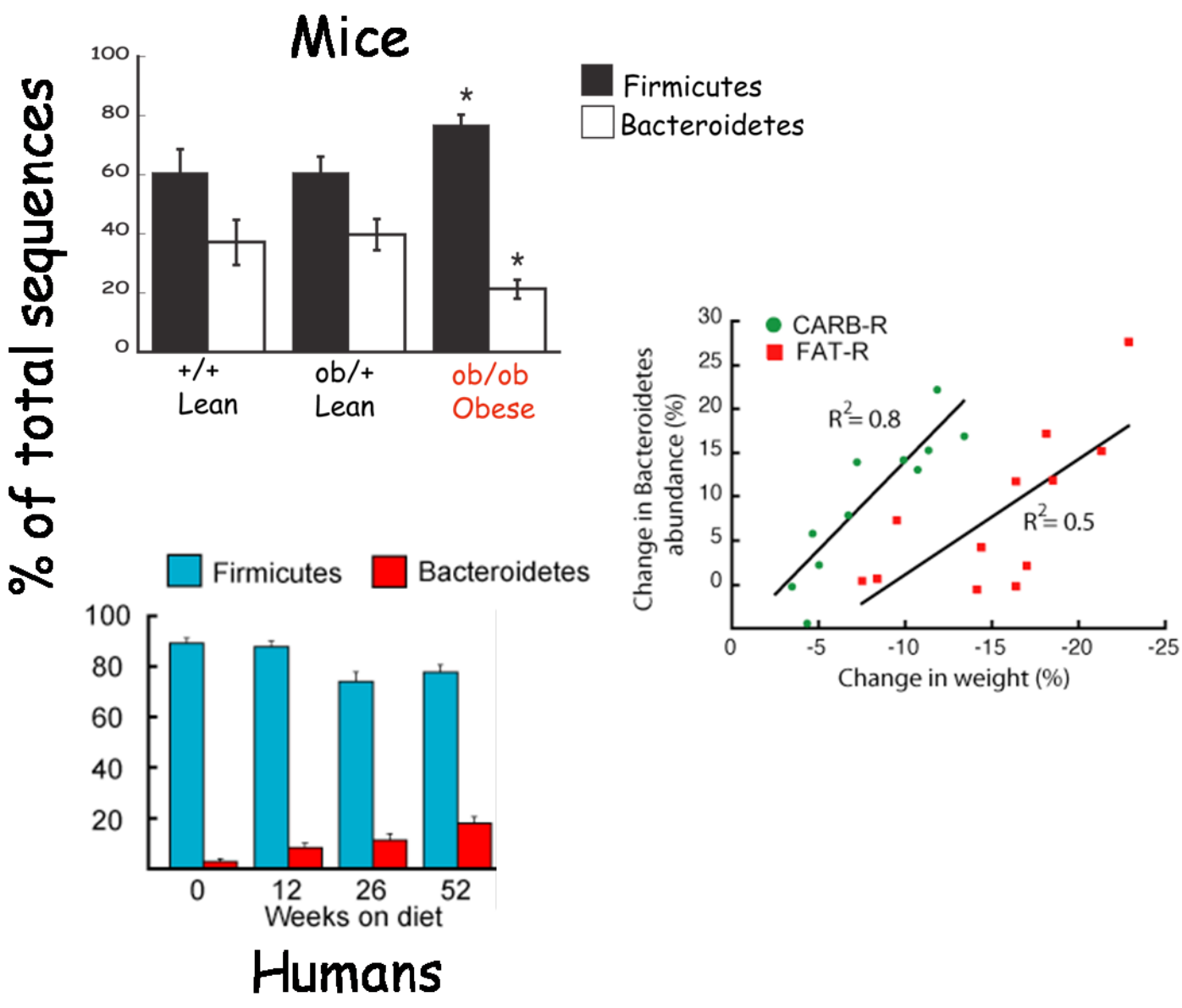
Males and females have equivalent responses

Studies of *Rag1-/-* mice: response does not require mature T- or B-cells



Conventionalized mice (CONV-D) = formerly germ-free (GF) recipients of a gut microbiota transplant from conventionally raised (CONV-R) donors

Link between adiposity and gut microbial

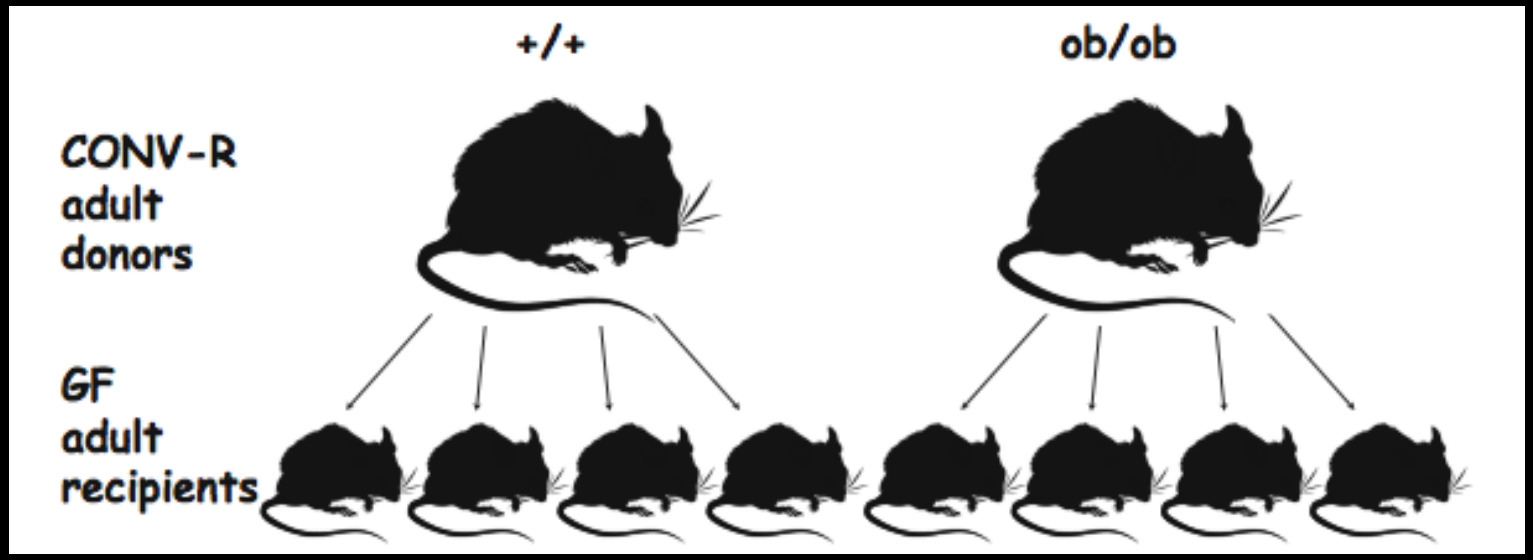


12 obese unrelated adult humans

FAT-R or CARB-R low calorie diets

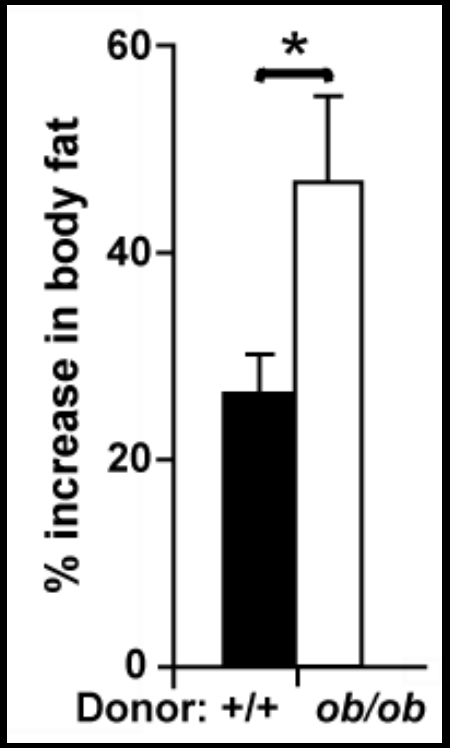
Each individual served as his/her control

Gut microbial community transplant experiments



Measured total body fat content before and after 14-day colonization

Increased adiposity phenotype is transmissible via the microbiota



Mice colonized with an 'obese' gut microbiota

Mice colonized with a 'lean' gut microbiota

No significant difference in chow consumption, initial body fat, or initial weight in the recipients

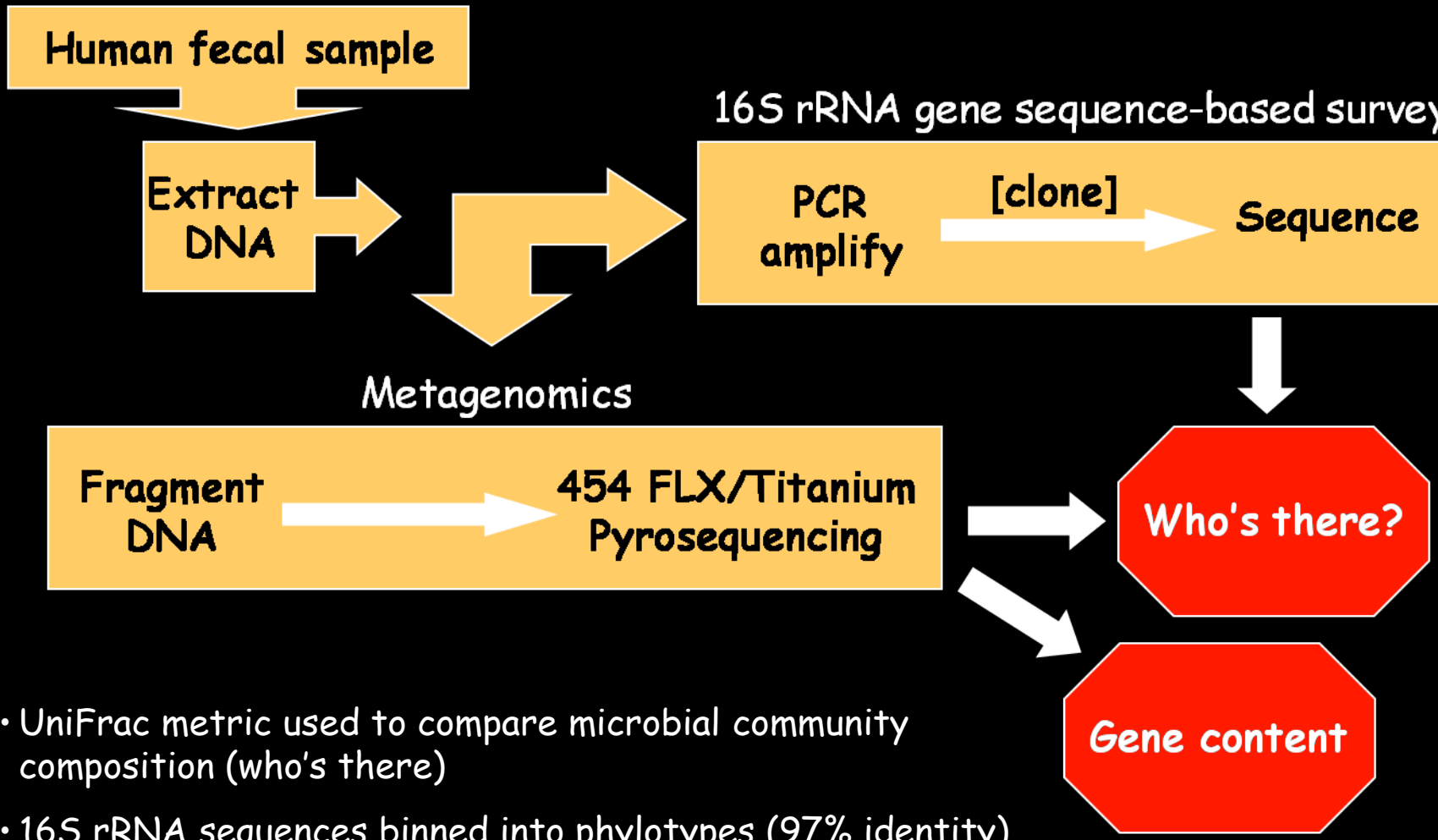
Also seen with gut communities transplanted from wt C57Bl/6J donors with diet-induced obesity

What role does shared environment, genotype, and obesity play in shaping the human gut microbiome?

Missouri Adolescent Female Twin Study (Birth Cohort)

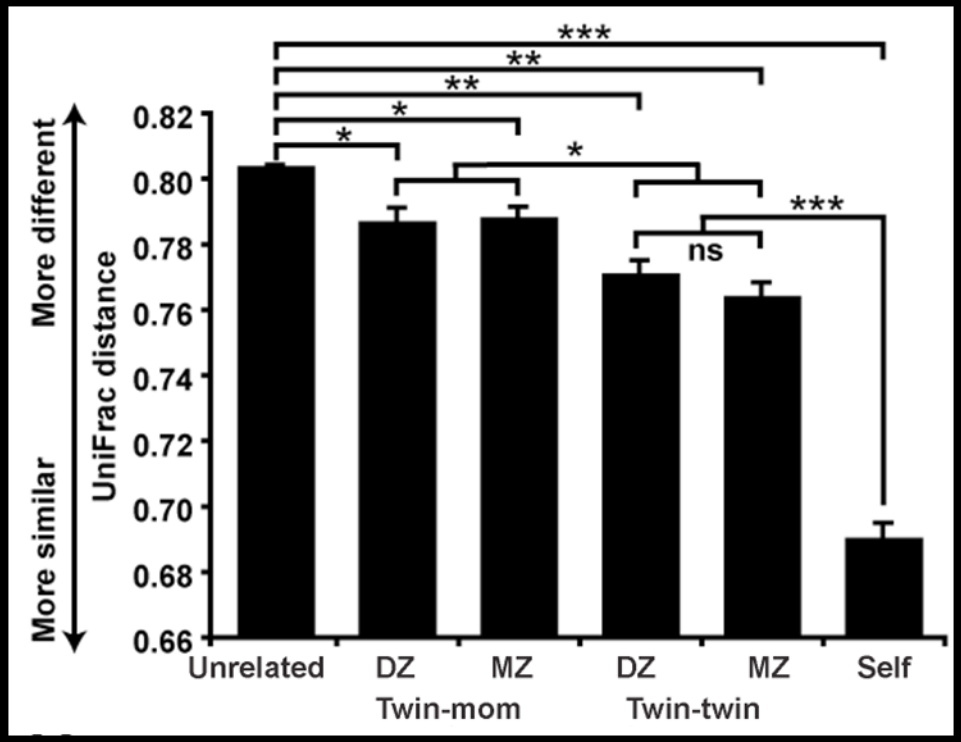
- 31 monozygotic (MZ) and 23 dizygotic (DZ) twin pairs recruited together with their mothers (46) (total of 154 individuals)
- Twins were 21-32 yrs old, European or African Ancestry, and concordant for obesity (BMI≥30kg/m^2) or leanness (BMI >18.5 <25kg/m^2)
- Participants broadly representative of Missouri population with respect to BMI, parity, education, marital status
- Range of distances between twins (30% of co-twins live together; twins distributed throughout USA)
- Fecal samples collected at t=0 and 2 months later

Basic workflow for characterizing gut microbial communities



- UniFrac metric used to compare microbial community composition (who's there)
- 16S rRNA sequences binned into phylotypes (97% identity)

The gut microbiota is more similar within families



*p<10^{-5}; **p<10^{-14}; ***p<10^{-41}

- Family members share significantly more phylotypes than unrelated individuals (*G*-test of OTU network, p<10^{-12})
- Covariance of MZ and DZ co-twin gut communities not significantly different; no discernible effect of degree of physical separation of co-twins
- Not a single abundant (>0.5% of population) species-level bacterial phylotype shared among all 154 individuals surveyed

Conclusions

- Early environmental exposures are a key determinant of adult gut microbial ecology (mothers and offspring share communities)
- Hypothesis that there is a core gut microbiome defined by abundant organismal lineages may be incorrect
- One possibility is that species-level variability results from extensive functional redundancy: i.e., different microbial communities (species assemblages) converge on the same functional state (similar functional gene repertoires)

Radically different species assemblages lead to similar functional profiles in macro-ecosystems…

Grasslands share many obvious similarities yet have none of their species in common

Highveld grassland near Heidelberg, South Africa

versus

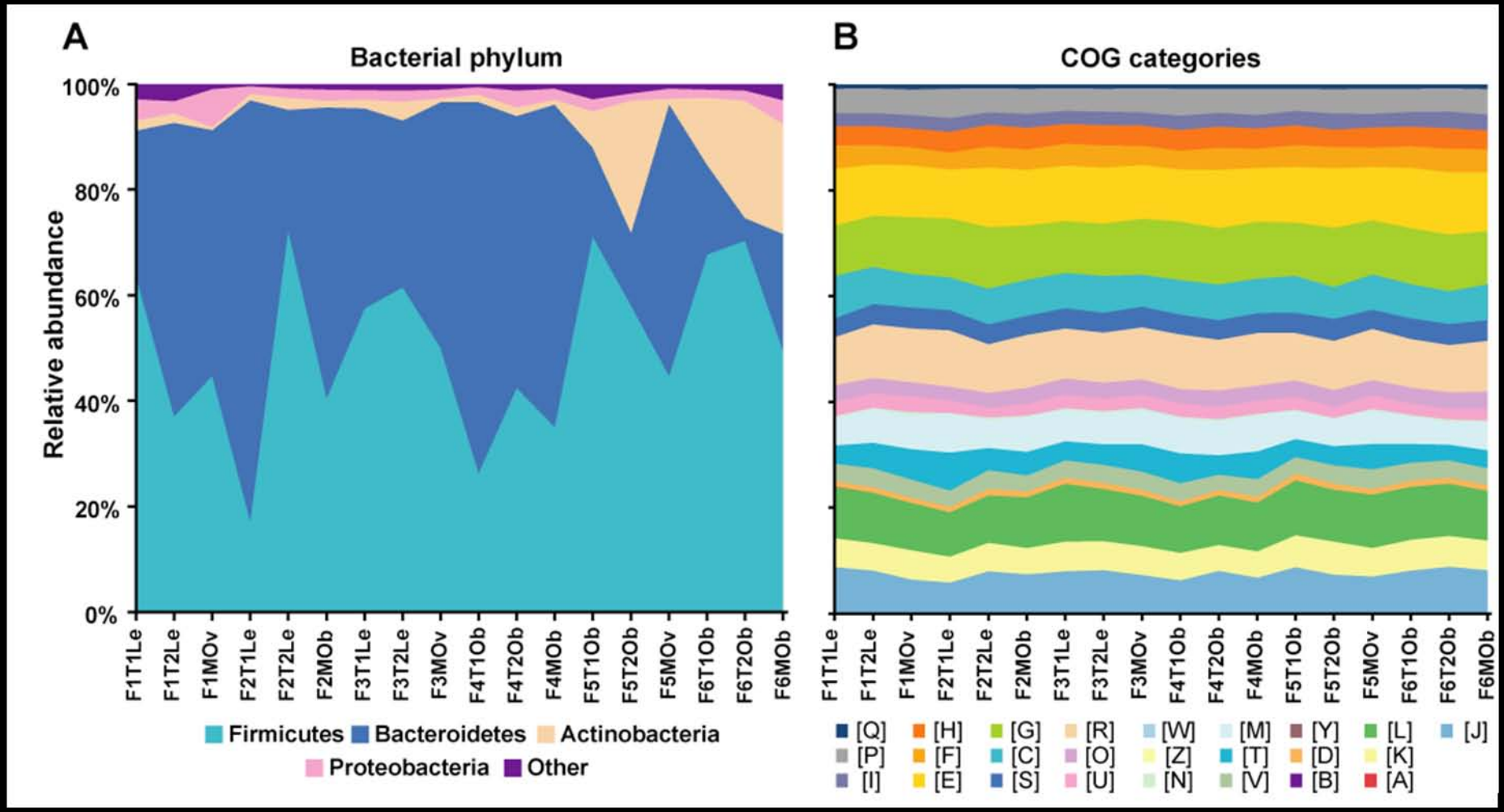
Tussock grassland, Central Otago, New Zealand

Tropical Rainforest, Bunga Forest,Zimbabwe

versus

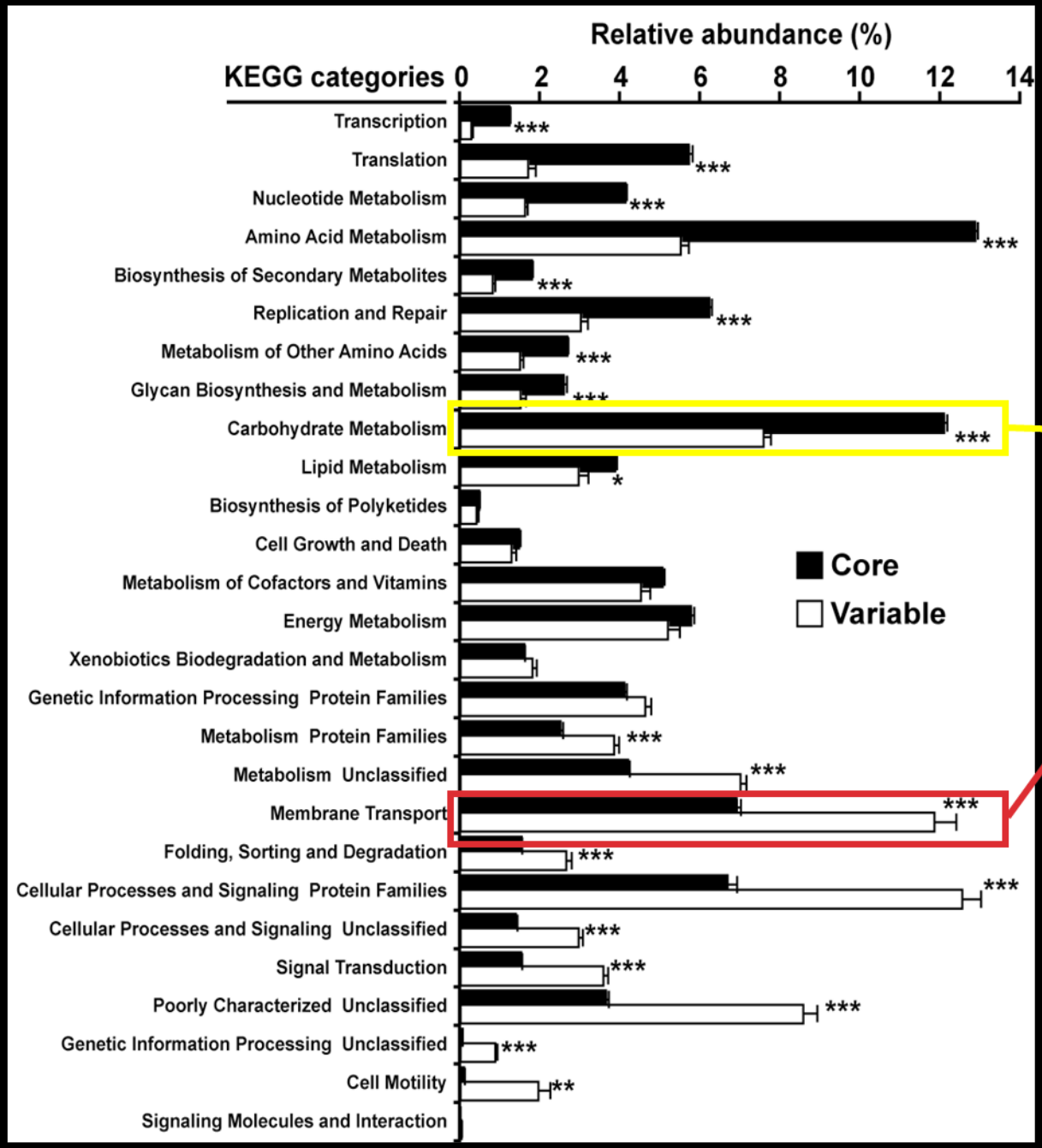
Temperate Rainforest, Fiordland, New Zealand

Different species assemblages lead to similar functional profiles in the gut



6 MZ twin pairs and their mothers

Functional analysis of gut microbiome (KEGG)



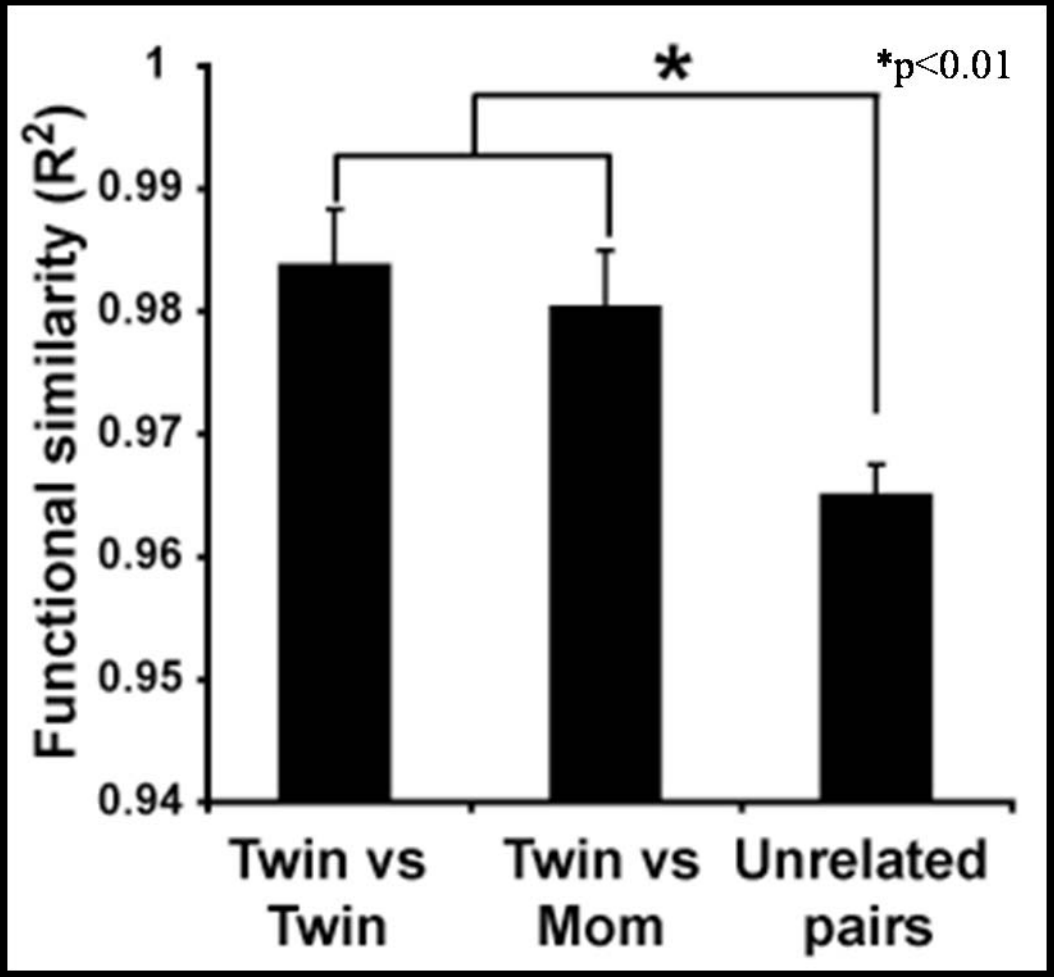
More carbohydrate metabolism in core

More membrane transport in variable

Sequences from each of the 18 microbiomes binned into 'core' or 'variable' microbiome based on the co-occurence of KOs ('core' groups found in all 18 microbiomes)

*p<0.05, **p<0.001, ***p<10^{-5}

Families have more functionally similar microbiomes



Average measure of functional similarity (based on pairwise comparison of metabolic profiles — the relative abundance of KEGG metabolic pathways)

Obesity-associated metabolic pathways and genes identified

- 383 genes as biomarkers of obesity (odds ratio >2 or <0.5 when comparing all obese microbiomes to the aggregate lean microbiome or vice versa): only non core-associated functional groups included in the comparison
- Validated by permuted t-test (q-value<0.05)
- Genes were representative of taxonomic differences between lean and obese gut communities (decrease in Bacteroidetes and increase in Actinobacteria/ Firmicutes)
- Many obesity-associated genes involved in carbohydrate metabolism (but amino acid and lipid metabolism also represented)

Looking ahead for the human microbiome project….

What constitutes a suitable reference control when attempting to correlate various physiologic or pathophysiologic states with host microbial ecology?

- A person is arguably his or her best control. A longitudinal (time course) study that relates changes in an individual's physiologic status to microbial community structure constitutes an attractive study design
- Attention needs to be devoted to characterizing the extent of intrapersonal variation that normally occurs in a microbiota occupying in a given body habitat when attempting to determine the effects of disease states
- Co-twin (concordant or discordant for physiologic or pathophysiologic state) or family members as other desirable controls

Looking ahead….

Need to perform experiments to learn more about how microbial communities operate

- Marriage of comparative metagenomics to transcriptomics, proteomics, *and metabolomics*
- Link to Protein Structure Initiative (reference genomes)
- Impact on our analyses of model organisms
- Renaissance in gnotobiology
  - creation of 'synthetic' communities; microbiota transplants
  - characterize the adaptations/evolution of communities within their habitats (exploring the world of ecogenomics)

Looking ahead….

Need for a global perspective about the human microbiome and HMP

- Explore the interface between our evolving cultures, technologies and our microbial ecology
- Human microbial observatories (HMOs!): looking back and forward; defining the evolution of our human 'metagenomes'
- Engineering microbial community metabolism for bioremediation and other needed host attributes (but important societal issues)
- *Dynamically evolving field* : (i) transformative time of democratization of genome sequencing; (ii) inherently interdisciplinary (interfaces = environmental/medical microbiology; evolutionary biology/ecology; chemistry/biology; systems biology/physiology/nutritional sciences; applied math/statistics; computational biology/CS; cultural anthropology/sociology/law)
- Think strategically, expansively,and creatively: use roadmap programs as a 'model organisms' (new educational programs - public and students; new technology spin-offs ?via SBIR program).